COMPARISON OF WATERLESS HAND ANTISEPSIS AGENTS AT SHORT APPLICATION TIMES: RAISING THE FLAG OF CONCERN

Sasi Dharan, MT; Stéphane Hugonnet, MD, MSc; Hugo Sax, MD; Didier Pittet, MD, MS

ABSTRACT

OBJECTIVE: Although alcohol-based hand rinses and gels have recommended application times of 30 to 60 seconds, healthcare workers usually take much less time for hand hygiene. We compared the efficacies of four alcohol-based hand rubs produced in Europe (hand rinses A, B, and C and one gel formulation) with the efficacy of the European Norm 1500 (EN 1500) reference waterless hand antisepsis agent (60% 2-propanol) at short application times.

DESIGN: Comparative crossover study.

SETTING: Infection Control Program laboratory of a large tertiary-care teaching hospital.

PARTICIPANTS: Twelve healthy volunteers.

INTERVENTION: Measurement of residual bacterial counts and log reduction factors following inoculation of fingertips with *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538, *Pseudomonas aeruginosa* ATCC 15442, and a clinical isolate of *Enterococcus faecalis*.

RESULTS: All hand rinses satisfied EN 1500 standards following a single application for 15 and 30 seconds, but reduction factors for the gel formulation were significantly lower for all tested organisms (all $P < .025$).

CONCLUSIONS: Under stringent conditions similar to clinical practice, all three hand rinses proved to be more efficacious than the marketed alcohol-based gel in reducing bacterial counts on hands. Further studies are necessary to determine the in vivo efficacy of alcohol-based gels and whether they are as efficacious as alcohol-based rinses in reducing the transmission of nosocomial infections (*Infect Control Hosp Epidemiol* 2003;24:160-164).

Nosocomial infections increase morbidity and mortality and strain the budgets of hospitals, but can be reduced by appropriate hand hygiene.[1-3] However, educating healthcare workers to adhere to hand hygiene recommendations is difficult and compliance remains low.[4] In the largest epidemiologic survey of hand hygiene practices,[5] time constraint was identified as the leading predictor for noncompliance during routine patient care, stressing the need for the use of fast-acting hand antisepsis agents.[5-7]

Hand hygiene has evolved significantly from the days when Semmelweis recommended chlorinated lime for hand antisepsis.[8] The use of alcohol-based hand rubs is currently recommended in certain healthcare facilities, or when caring for patients colonized or infected with highly resistant organisms.[3-7,9-11] A new guideline for hand hygiene in healthcare settings recommends their use for routine hand decontamination in most clinical situations.[12] Rubbing hands with alcohol-based agents eliminates some of the inconveniences related to doing so with plain or antimicrobial soaps as these agents act faster, have proven broad-spectrum activity, and can be made immediately available at the patient's bedside, thus resulting in increased use and enhanced impact.[3,7,9,10,12,13] Early formulations of alcohol-based solutions had the disadvantage of drying the skin. This has been overcome by the incorporation of emollients into gels or rinses, or the adjunct use of hand-moisturizing creams.[14,15]

Alcohol-based hand rinses and gels have recommended application times of 30 to 60 seconds,[9,12] but, on the basis of direct observations, the time taken by nursing staff for hand washing actually ranges from 6 to 24 seconds.[12] However, few peer-reviewed studies have evaluated the efficacy of short application times for alcohol-based hand rub formulations.[16,17] We developed a method to study this issue and assessed the efficacies of three alcohol-based hand rinses and one gel, all of which met official standards.[18]

METHODS

Twelve healthy volunteers who had no visible injuries to their hands and who had not used hand antisepsis for at least 24 hours previously were enrolled in the study, which was conducted in the laboratory of the Infection Control Program at the University of Geneva Hospitals. The method used was a modification of the European Norm 1500 (EN 1500).[18]

Briefly, EN 1500 requires 12 to 15 test subjects and

The authors are from the Infection Control Program, Department of Internal Medicine, University of Geneva Hospitals, Geneva, Switzerland.
Address reprint requests to Professor Didier Pittet, Director, Infection Control Program, Department of Internal Medicine, University of Geneva Hospitals, 1211 Geneva 14, Switzerland.
Supported in part by educational grants from Bode Chemie GmbH, Hamburg, Germany; and Blue Skin, Paris, France.
The authors thank R. Sudan for editorial assistance.

a 24-hour broth culture of *Escherichia coli* K12 (a nonvirulent strain). Hands are washed with a soft soap and dried. Both hands are immersed halfway to the metacarpals in the broth culture for 5 seconds. They are removed, excess fluid is drained off, and they are dried in the air for 3 minutes. Bacteria are recovered for the initial count by kneading the fingertips of each hand separately in 10 mL of trypticase soy broth (Becton Dickinson, Cockeysville, MD) without neutralizers for 60 seconds. The hands are removed and disinfected with 3 mL of the hand antisepsis agent for 30 seconds in a set design.

The same procedure is repeated with a further application of 3 mL of the agent with a total disinfection time not exceeding 60 seconds. Both hands are rinsed in running water for 5 seconds, and excess water is drained off. The fingertips of each hand are kneaded separately in 10 mL of trypticase soy broth with added neutralizers. These broths are used to obtain the final count.

Log_{10} dilutions of recovery medium are prepared and plated on trypticase soy agar. Colony counts are performed after 24 and 48 hours of incubation at 36°C. The average colony count of the two hands is used for evaluation. The log reduction factors (RFs) are calculated and compared between the initial count and the final count. Within 3 hours, with the use of the same broth inoculum, all test subjects should be tested with the reference hand rub (60% 2-propanol) and the product to be tested. The RF of the hand antisepsis agent being tested should be superior to or the same as that of the reference alcohol-based rub for acceptance. If the RF of the test product is inferior, then the results are analyzed statistically by the Wilcoxon matched pairs signed rank test. If the difference is significant ($P < .05$), the product is not acceptable.

The choice of organisms, hand contamination, hand disinfection, bacterial recovery, and culture medium were modified with this method. Bacteria tested included *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538, *Pseudomonas aeruginosa* ATCC 15442, and a clinical isolate of *Enterococcus faecalis*. As stipulated in EN 1500, 60% 2-propanol (vol/vol) was used as the reference antisepsis hand rub.[18] Products tested were hand rinses A, B (Bode Chemie GmbH, Hamburg, Germany), and C (in-house preparation)[3] and an alcohol-based hand gel (Blue Skin, Paris, France). Investigators were blinded to the composition of rinses A and B.

The active substances of the hand rubs were as follows: A, 80% ethanol (wt/vol); B, 95% ethanol (wt/vol); C, 75% isopropanol (vol/vol) and chlorhexidine gluconate (0.5%); and gel, 60% isopropanol.

Two study participants were tested daily with one bacterial strain for the products and the reference hand rub. Hands were washed with disinfectant-free soap and dried using paper towels. The left hand was used for the control, and the right hand for the test. The palmar surface of the fingertips of both hands was contaminated by placing 10 µL of overnight broth culture of organisms on each fingertip and allowing it to dry for 2 minutes by gentle rubbing of the thumb against each fingertip. Control fingertips (left hand) were then immersed in 10 mL of trypticase soy broth and kneaded for 30 seconds. This sample was processed to obtain the initial count. Hand rub solution (0.4 mL) was applied to the cupped fingertips of the right hand, which were disinfected by rubbing of the thumb against fingertips and fingernails. Disinfecting times were 15 and 30 seconds.

TABLE 1
ANTIMICROBIAL EFFICACY OF HAND RUBS WITH SHORT APPLICATION TIMES FOR *STAPHYLOCOCCUS AUREUS*

	Mean log_{10} (N = 12)			
Product	Initial Count	Final Count	Reduction Factor	*P*
60% 2-propanol (15 s)	7.65	1.75	5.90	
Rinse A (15 s)	7.63	1.47	6.15	.308
Rinse B (15 s)	7.77	1.95	5.82	.583
Rinse C (15 s)	7.60	2.15	5.45	.530
Gel (15 s)	7.72	3.62	4.10	.005
60% 2-propanol (30 s)	7.62	1.26	6.36	
Rinse A (30 s)	7.74	0.86	6.88	.209
Rinse B (30 s)	7.79	1.46	6.33	.875
Rinse C (30 s)	7.61	0.84	6.78	.272
Gel (30 s)	7.37	2.34	5.03	.012

Trypticase soy broth was used as sampling fluid and diluent. The following neutralizers were added to trypticase soy broth when sampling after disinfection: polysorbate 80 (30 mL/L), saponin (30 g/L), histidine (1 g/L), cystine (1 g/L), and lecithin (3 g/L). Trypticase soy agar (Becton Dickinson) plus 5% sheep blood was used for enumeration of bacteria.

Log_{10} dilutions of the sampling fluids were performed immediately in trypticase soy broth (10^{-1} to 10^{-6} for the control samples and 10^{-2} for the test samples). For the control samples, 0.1 mL of 10^{-4} to 10^{6} dilutions were plated in duplicate on blood agar. For the test samples, 1 mL and 0.1 mL of neat sampling fluids and 0.1 mL of the diluted fluids were plated in duplicate. All plates were incubated overnight at 36°C. The colony count was then performed and the plates were reincubated for another 24 hours.[18]

Final colony counts were performed at 48 hours. The number of colony-forming units (CFU) per milliliter of sampling fluid was calculated and transformed to a decimal logarithm.[18] For computational reasons, test values of 0 were set at 1.

The measure of interest was the RF (RF: log initial CFU – final CFU) calculated for each organism, product, and disinfection time.[17] Differences were tested using the Wilcoxon matched pairs signed rank test (two-sided) for statistical significance ($P \leq .05$). For each product and disinfection time, 12 values were available for analysis.

RESULTS

The initial counts obtained following hand inoculation were homogeneous in all series and did not differ

TABLE 2
ANTIMICROBIAL EFFICACY OF HAND RUBS WITH SHORT APPLICATION TIMES FOR *ENTEROCOCCUS FAECALIS*

Product	Mean $\log_{10}$ (N = 12) Initial Count	Final Count	Reduction Factor	*P*
60% 2-propanol (15 s)	7.53	2.53	5.03	
Rinse A (15 s)	7.52	1.59	5.94	.012
Rinse B (15 s)	7.54	1.76	5.78	.013
Rinse C (15 s)	7.59	2.31	5.28	.583
Gel (15 s)	7.59	3.93	3.66	.003
60% 2-propanol (30 s)	7.47	1.40	6.07	
Rinse A (30 s)	7.53	0.90	6.63	.005
Rinse B (30 s)	7.55	1.25	6.29	.117
Rinse C (30 s)	7.70	0.75	6.95	.008
Gel (30 s)	7.20	2.30	4.90	.023

TABLE 3
ANTIMICROBIAL EFFICACY OF HAND RUBS WITH SHORT APPLICATION TIMES FOR *PSEUDOMONAS AERUGINOSA*

Product	Mean $\log_{10}$ (N = 12) Initial Count	Final Count	Reduction Factor	*P*
60% 2-propanol (15 s)	7.29	1.24	6.05	
Rinse A (15 s)	7.29	0.50	6.80	.071
Rinse B (15 s)	7.16	0.95	6.21	.347
Rinse C (15 s)	7.96	1.97	5.99	.754
Gel (15 s)	7.89	3.74	4.15	.003
60% 2-propanol (30 s)	7.23	0.42	6.81	
Rinse A (30 s)	7.21	0.33	6.88	.433
Rinse B (30 s)	7.29	0.65	6.63	.308
Rinse C (30 s)	7.97	0.79	7.18	.126
Gel (30 s)	7.25	2.09	5.16	.005

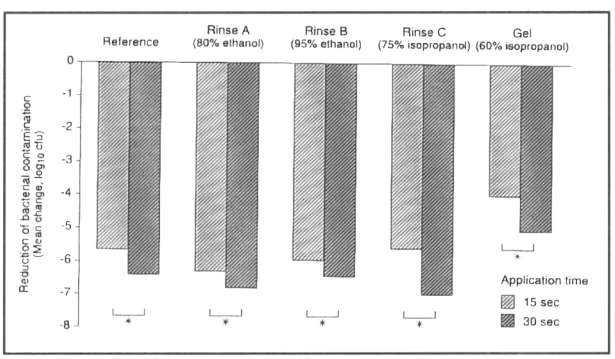

FIGURE. Comparison of the antimicrobial efficacies of hand rub formulations after 15 and 30 seconds of application. Reduction factors at 15 and 30 seconds for the three organisms tested (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) were compared statistically in paired samples. **P* values less than .01. cfu = colony-forming units.

according to the products or organisms tested (data not shown; average CFU are listed in Tables 1–3). The average bacterial counts following hand rinse application (final counts) and RFs with a disinfecting time of 30 seconds were all satisfactory. In particular, bacterial reduction reached at least 5 logs after rinse application (Tables 1–3). In contrast, after 30 seconds of gel application, the residual bacterial contamination was significantly higher and the RF significantly lower compared with the reference hand rub.

The residual contamination was significantly lower and the RF significantly higher (Figure) following longer application of both test and reference hand rub formulations. However, with the exception of the gel, both the residual bacterial counts and the RFs satisfied EN 1500 standards for clinical application. After 15-second applications, the bacterial counts and observed RFs for all products except the gel satisfied EN 1500 standards (Tables 1–3).

The antimicrobial efficacies of the tested rinses (A, B, and C) against *Enterococcus faecalis* were higher than that of the reference standard after 15 and 30 seconds (Table 2); they reached statistical significance for A and B with a disinfection time of 15 seconds and for A and C after 30 seconds.

DISCUSSION

Following the single application of the three hand rinses tested, residual bacterial contamination and reduction met required standards (ie, 5-$\log_{10}$ reduction) after 15 seconds. Increasing the time to 30 seconds further enhanced antibacterial activity (Figure). The rinses performed significantly better than the gel tested, which failed to meet the criteria for use for all tested organisms after both 15 and 30 seconds of application.

The alcohol-based gel was significantly less efficacious than both the test rinses and the reference hand rub; this could be due to the active substance not being readily available in a short time.[9] Notably, the hands were often wet after 15 seconds of gel application. This hypothesis should be evaluated further with faster-drying gels or by increasing the alcohol content of the product.[19] However, on the basis of our results, the gel tested cannot be recommended for use in European hospitals.

In a prospective randomized clinical trial recently conducted in an intensive care setting,[20] Larson et al. found that the use of an alcohol-based hand rub was less damaging to healthcare workers' hands, saved time, was less expensive, and was at least as efficacious as a conventional antiseptic wash containing chlorhexidine gluconate. Importantly, the time required for hand hygiene averaged 12.7 seconds when the hand rub was used compared with 21.1 seconds for the antiseptic wash ($P \leq .01$), confirming that the time taken by healthcare personnel for hand hygiene is rather short,[12] especially when workloads are high[3,5] and the setting is critical care.[13] The aforementioned advantages tend to suggest that less time-

consuming hand rubbing should replace standard hand washing practices,[3,6,7,10,12,13,20,21] thus overcoming the barrier of time constraints.[13]

However, hand rub solution must be highly efficacious at short times. Current data suggest that shortening the duration of rinse application from 30 to 15 seconds is associated with a trend toward reduced antimicrobial efficacy, and that the use of the tested hand gel is clearly less efficacious at both times. Further testing of hand hygiene agents at the bedside using standardized protocols to obtain more realistic views of microbial colonization and risk of bacterial transfer and cross-transmission will certainly be necessary.[22] Furthermore, whether results of laboratory experiments after hand contamination could be translated into an estimation of the risk of cross-transmission in clinical practice remains to be tested in controlled clinical trials.

Because alcohols have excellent activity and the most rapid bactericidal action of all antiseptics, they are the preferred agents for waterless hand antisepsis.[12] Their antimicrobial activity is due to their ability to denature proteins.[9] Solutions containing 50% to 80% alcohol are most efficacious, with higher concentrations being less potent. This paradox is due to the fact that proteins are not denatured easily in the absence of water. Given that rinses A and B differed only in their ethanol content (80% vs 95%, wt/vol, respectively), they were compared with each other (data not shown). Although none of the comparisons showed any statistical significance, a trend toward a higher antibacterial activity of A over B was observed. With this study, we concluded that solutions containing 70% to 80% alcohol are better suited for hand disinfection with short contact times.

Because alcohol alone does not have any lasting effect, another antiseptic agent is sometimes added to the formulation to prolong its activity[9,15,22]; in this study, rinse C contained chlorhexidine gluconate in addition to isopropanol. As indicated by our results and as expected,[9] the chlorhexidine did not provide a significant additional benefit at short application times, which was in contrast to our results in different clinical conditions in which testing was performed after much longer times.[22]

EN 1500 is the method most widely used in Europe to evaluate the efficacy of hand hygiene agents.[18] In the United States, antiseptic hand wash products intended for use by healthcare personnel are regulated by the Food and Drug Administration's Division of OTC Drug Products and evaluated using a standardized method (E 1174-00).[23] Tests are performed in accordance with the manufacturer's instructions for the test material and test organism (usually *Serratia marcescens*) using the glove juice technique.[12,23] Application time is 30 seconds followed by rinsing with tap water for 30 seconds. Shorter hand washing times (eg, 15 seconds) are permitted if elected by manufacturers.

For waterless formulations, a similar procedure employing 5 mL of test material is used. Thresholds for acceptance are (1) a 2-$\log_{10}$ reduction of the test organism within 5 minutes after the first wash, and (2) a 3-$\log_{10}$ reduction within 5 minutes after the tenth wash; there is no reference standard for comparison in contrast to EN 1500 requirements.[12,23] Ethanol 60% to 95% and povidone-iodine 5% to 10% are approved for antiseptic hand washes by the U.S. Food and Drug Administration.

With our modified method we tested potential pathogens rather than only *Escherichia coli* K12 and showed that different alcohol-based formulations have different killing rates against different bacteria. The three hand rinses tested were at least as efficacious as the reference hand rub for degerming hands. A higher antibacterial activity was observed for the rinses, particularly under conditions of stress: shorter times for hand disinfection and organisms that were more difficult to kill (eg, *Enterococcus faecalis*). The relevance of this observation needs further testing in controlled clinical conditions.

The method used, particularly the short disinfection time, more closely resembles actual product use in patient care. It offers the potential to perform comparisons between hand hygiene agents to assist in the development of more effective strategies to decrease the spread of nosocomial pathogens. Under stringent conditions similar to clinical practice, both currently used and newly designed hand rinses were of greater efficacy than a marketed alcohol-based gel.

The major limitation of this study is that only one gel was tested. Because gel formulations differ in the type and amount of alcohol present and in additional components, it cannot be assumed that the results of laboratory-based tests of the efficacy of one product will be representative of all products. Recently published data suggest that further research is needed to develop alcohol-based hand gels with antibacterial efficacy that is at least similar to that of most rinses available on the market.[19]

On the basis of the current data, before alcohol-based gels can be recommended for use in European hospitals, studies must be conducted to determine whether they are as effective as rinses in reducing cross-transmission of nosocomial pathogens. Although alcohol-based hand rubs have not been widely introduced in U.S. hospitals, they should be considered for implementation because of their multiple advantages.[3-7,9-13,15,20,21,24,25]

As recently emphasized,[24-26] the choice between rinse and gel should be based not only on antimicrobial efficacy, but also on healthcare workers' acceptance and tolerance of the product and the institution's experience. This is absolutely essential to ensure widespread and appropriate use at the bedside with the ultimate objective being improved patient safety.[3]

REFERENCES

1. Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients: excess length of stay, extra costs and attributable mortality. *JAMA* 1994;271:1598-1601.
2. Simmons B, Bryant J, Neiman K, Spencer L, Arheart K. The role of handwashing in prevention of endemic intensive care unit infections. *Infect Control Hosp Epidemiol* 1990;11:589-594.
3. Pittet D, Hugonnet S, Harbarth S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *Lancet* 2000;356:1307-1312.

4. Pittet D. Improving compliance with hand hygiene in hospitals. *Infect Control Hosp Epidemiol* 2000;21:381-386.
5. Pittet D, Mourouga P, Perneger TV, Infection Control Program. Compliance with handwashing in a teaching hospital. *Ann Intern Med* 1999;130:126-130.
6. Boyce JM. It is time for action: improving hand hygiene in hospitals. *Ann Intern Med* 1999;130:153-155.
7. Voss A, Widmer AF. No time for handwashing!? Handwashing versus alcoholic rub: can we afford 100% compliance? *Infect Control Hosp Epidemiol* 1997;18:205-208.
8. Semmelweis I. *Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers.* Pest, Wien und Leipzig: C. A. Hartleben's Verlag-Expedition; 1861:1-544.
9. Rotter M. Hand washing and hand disinfection. In: Mayhall CG, ed. *Hospital Epidemiology and Infection Control,* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1999:1339-1355.
10. Larson E. Skin hygiene and infection prevention: more of the same or different approaches? *Clin Infect Dis* 1999;29:1287-1294.
11. Zaragosa M, Salles M, Gomez J, Bayas JM, Trilla A. Handwashing with soap or alcoholic solutions? A randomised clinical trial of its effectiveness. *Am J Infect Control* 1999;27:258-261.
12. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America. *MMWR* 2002;51(RR-16):1-45.
13. Hugonnet S, Perneger TV, Pittet D. Alcohol-based handrub improves compliance with hand hygiene in intensive care units. *Arch Intern Med* 2002;162:1037-1043.
14. Rotter ML, Koller W, Neumann R. The influence of cosmetic additives on the acceptability of alcohol-based hand disinfectants. *J Hosp Infect* 1991;18:57-63.
15. Boyce JM, Kelliher S, Vallande N. Skin irritation and dryness associated with two hand hygiene regimens: soap and water handwashing versus hand antisepsis with an alcoholic hand gel. *Infect Control Hosp Epidemiol* 2000;21:442-448.
16. Frantz SW, Haines KA, Azar CG, Ward JI, Homan SM, Roberts RB. Chlorhexidine gluconate (CHG) activity against clinical isolates of vancomycin-resistant *Enterococcus faecium* (VREF) and the effects of moisturizing agents on CHG residue accumulation on the skin. *J Hosp Infect* 1997;37:157-164.
17. Ayliffe GAJ, Babb JR, Quoraishi AH. A test for "hygienic" hand disinfection. *J Clin Pathol* 1978;31:923-928.
18. European Committee for Standardization. *EN 1500, Chemical Disinfectants and Antiseptics—Hygienic Handrub: Test Method and Requirements (Phase 2, Step 2).* Brussels, Belgium; 1997.
19. Kramer A, Rudolph P, Kampf G, Pittet D. Limited efficacy of alcohol-based hand gels. *Lancet* 2002;359:1489-1490.
20. Larson E, Aiello A, Bastyr J, et al. Assessment of two hand hygiene regimens for intensive care unit personnel. *Crit Care Med* 2001;29:944-975.
21. Trick W, Weinstein R. Hand hygiene for intensive care unit personnel: rub it in. *Crit Care Med* 2001;29:1083-1084.
22. Pittet D, Dharan S, Touveneau S, Sauvan V, Perneger TV. Bacterial contamination of the hands of hospital staff during routine patient care. *Arch Intern Med* 2000;159: 821-826.
23. American Society for Testing Materials. *ASTM E 1174-00: Standard Test Method for Evaluation of Health Care Personnel or Consumer Handwash Formulations.* West Conshohocken, PA: American Society for Testing Materials; 2000.
24. Boyce J. Antiseptic technology: access, affordability and acceptance. *Emerg Infect Dis* 2001;7:231-233.
25. Pittet D. Improving adherence to hand hygiene practice: a multidisciplinary approach. *Emerg Infect Dis* 2001;7:234-240.
26. Pittet D, Kramer A. Alcohol-based hand gels and hand hygiene in hospitals: author reply. *Lancet* 2002;360:1511.